AIR FORCE HEALTH STUDY

(2)

AN EPIDEMIOLOGIC INVESTIGATION OF HEALTH EFFECTS IN AIR FORCE PERSONNEL FOLLOWING EXPOSURE TO HERBICIDES

EXTRACT
REPRODUCTIVE OUTCOMES
EXECUTIVE SUMMARY
INTRODUCTION AND CONCLUSIONS
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This report summarizes the findings of an investigation of reproductive outcomes of the 791 Ranch Hands and 942 Comparisons for whom a dioxin level had been determined by August, 1991. These men have fathered 5,489 pregnancies including 4,514 live births. These men are a subset of all Ranch Hands (n=1,098) and Comparisons (n=1,549) who have fathered 8,263 pregnancies and 6,792 live births. All data in this report have been verified by review of birth certificates, newborn clinic records, health records and death certificates. The birth defect status of each child was verified through the age of 18.

This study is the first to combine a direct measurement of dioxin level with documented and verified reproductive outcomes in a population of sufficient size to provide a reasonable opportunity to detect associations between paternal dioxin levels and a range of common reproductive outcomes. This study has good statistical power to detect relative risks of 2 or greater for common birth defects such as musculoskeletal deformities but low statistical power for relative risks of this order for rare conditions such as chromosomal abnormality or infant death.

Three dioxin measures were used: the extrapolated initial dose, current dioxin with adjustment for time since departure from SEA, and categorized current dioxin. The first two of these models were applied only to conceptions and children of Ranch Hands. The third included conceptions and children of both Ranch Hands and Comparisons.

The few positive associations found between dioxin and reproductive outcomes were generally weak, inconsistent or biologically implausible. These data provide no support for the hypothesis that paternal dioxin exposure is adversely associated with reproductive outcomes. Whether dioxin exposure of the mother before or during pregnancy results in abnormalities in the developing fetus or child could not be addressed in this study and remains an open question.

EXECUTIVE SUMMARY

The Air Force is conducting a 20-year prospective study of the health of veterans of Operation Ranch Hand, the unit responsible for aerial spraying of herbicides in Vietnam from 1962 to 1971. The health of those veterans is compared to that of a group of other Air Force veterans who served in Southeast Asia (SEA) and who had no occupational exposure to herbicides. The study, called the Air Force Health Study (AFHS), is in its tenth year and is designed to determine whether exposure to the herbicides or their contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin), has adversely affected the health, survival or reproductive outcomes of Ranch Hands.

This report summarizes the findings of an investigation of reproductive outcomes of the 791 Ranch Hands and 942 Comparisons for whom a dioxin level had been determined by August, 1991. These men have fathered 5,489 pregnancies including 4,514 live births. These men are a subset of all Ranch Hands (n=1,098) and Comparisons (n=1,549) who have fathered 8,263 pregnancies and 6,792 live births. All data in this report have been verified by review of birth certificates, newborn clinic records, health records and death certificates. The health status of each child was verified through the age of 18.

The analysis of birth defects in the baseline AFHS report, released in 1984, found that the Ranch Hand rate of reported pre-SEA defects was less than the Comparison rate and the Ranch Hand rate of reported post-SEA defects was greater than the Comparison rate. The baseline finding motivated the verification of conception outcomes and birth defects which are the subject of this report. Reanalysis using verified data also found similar results; however, additional analyses found no indication that these group differences were related to paternal dioxin levels. Furthermore, analyses within each of 13 categories of birth defects found no evidence that this finding was attributable to any specific category of anomalies.

This study is the first to combine a direct measurement of dioxin level with documented and verified reproductive outcomes in a population of sufficient size to provide a reasonable opportunity to detect possible associations between paternal dioxin levels and a range of common reproductive outcomes. This study has good statistical power to detect relative risks of 2 for common birth defects such as musculoskeletal deformities but low statistical power for relative risks of 2 for rare conditions such as chromosomal abnormality or infant death.

Three types of analyses that compared reproductive outcomes to dioxin levels are presented here. Dioxin levels were measured in 1987, and in one analyses those measurements were used to estimate initial doses received in SEA. Current dioxin levels with adjustment for time since departure from SEA, and current categorized dioxin levels were used in the other two analyses.

Analyses of miscarriage, total adverse outcome, total conceptions, birth weight, birth defects, birth defect severity, specific birth defects, infant death and neonatal death were carried out on all conceptions and children and with restriction to full siblings (step-children were excluded) to minimize genetic variation. Additionally, all reproductive outcomes except sperm count, percent abnormal sperm and multiple birth defects were analyzed with and without consideration of the pre-SEA reproductive experiences of these men.

Verification of all live births and conceptions revealed that Ranch Hands and Comparisons misreported birth defects similarly. About 2% of all pre-SEA and post-SEA children had parent-reported birth defects that could not be verified. Both groups under-reported 7% of pre-SEA defects and 14% of post-SEA defects.

Semen

The association between the father's dioxin level and sperm count and the percentage of abnormal sperm was assessed based on semen specimens collected during the baseline examination in 1982. No significant association was found between dioxin and sperm count or the percentage of abnormal sperm.

Miscarriage, Total Adverse Outcome and Total Conceptions

Analyses of miscarriage adjusted for the outcomes of pre-SEA pregnancies were generally negative. Although miscarriages increased with dioxin in conceptions fathered by Ranch Hands with late tours, they decreased in those with early tours. Since it seems implausible that dioxin would act differently in the two groups, it is concluded that dioxin does not affect miscarriage rates. Furthermore, the highest number of post-SEA conceptions was found in Ranch Hands having the highest dioxin levels, which argues against a relationship between dioxin exposure and miscarriage.

Similar to the results obtain for miscarriage, the rate of adverse outcomes increased with dioxin in Ranch Hands with early tours and decreased in Ranch Hands with late tours. Like those for miscarriage, these findings don't make biologic sense and appear unrelated to dioxin.

Birth Weight

Analyses of birth weight with adjustment for birth weights of pre-SEA children were mostly negative. The few significant findings were not suggestive of a dioxin effect. Among pre-SEA children, the rate of abnormally low birth weight in children of Ranch Hands with the lowest dioxin levels (61.2 per 1000) was less than that in children of Comparisons (73.5 per 1000) and in

post-SEA children, the rate in Ranch Hand children (93.3 per 1000) was greater than that in children of Comparisons (41.9 per 1000). This change is due as much to the decrease in the Comparison rate as to the increase in the Ranch Hand rate.

Analyses of birth weight without statistical adjustment for birth weights of pre-SEA children were generally negative or were complicated by interactions with covariates that lack biological explanation. After restriction to full siblings, birth weight decreases with dioxin in some strata and increases in others, suggesting that these findings are chance occurrences. We find no evidence in these data to suggest that birth weight is adversely associated with the father's dioxin level.

Birth Defects

The significance of the association between paternal dioxin level and birth defects was assessed within each of 13 categories of anomalies (total congenital, nervous system, eye, ear face and neck, circulatory system and heart, respiratory system, digestive system, genital, urinary, musculoskeletal, skin, chromosomal and other unspecified). Analyses were first conducted on all children and then with restriction to full sibling children. Each analysis was carried out first without and then with adjustment for covariates.

Few significant associations were found. Those that were found did not appear consistently across analyses and most were not suggestive of a plausible dioxin effect. Some analyses of total congenital anomalies and musculoskeletal deformities found significant relative risks, but no consistent patterns emerged. For example, an analysis of total congenital anomalies found that children of Ranch Hand officers with low dioxin levels had a lower anomaly rate than children of Comparisons. Children of Ranch Hand enlisted flyers and enlisted ground personnel with low dioxin levels had higher rates than children of Comparisons, but the rates in children of fathers with the highest dioxin levels were not elevated. These findings are consistent with the conclusion that the apparent associations are chance occurrences and that there is no underlying association between paternal dioxin and birth defects.

Birth Defect Severity

No consistent pattern of association between birth defect severity and dioxin levels was found. For instance, in some analyses, the highest rates were found in children born to Ranch Hands with intermediate dioxin levels, while the lowest rates were found in children born to Ranch Hands with the highest dioxin levels. We conclude that there is no evidence in these data to suggest that dioxin is adversely associated with birth defect severity.

Specific Birth Defects and Developmental Anomalies

Twelve specific birth defects (anencephaly, spina bifida, hydrocephalus, cleft palate, cleft lip/palate, esophageal atresia, anorectal atresia, polydactyly, limb reduction defects, hypospadias, congenital hip dislocation, Down's syndrome) and 4 developmental anomalies (disturbance of emotion, hyperkinetic syndrome of childhood, specific delays in development, mental retardation) were investigated. Of these, there were only enough occurrences of specific delays in development and hyperkinetic syndrome of childhood to permit statistical analysis.

Analyses of hyperkinetic syndrome with pre-SEA adjustment were entirely negative. Two unadjusted analyses of specific delays in development found significant associations but these were not supportive of a hypothesis of adverse effects of dioxin. One of these findings was caused by decreasing rates with extrapolated initial dioxin. The other was due to high post-SEA rates in children of Ranch Hands with intermediate dioxin levels and lower rates in children of Ranch Hands with high dioxin levels. Analyses of hyperkinetic syndrome without pre-SEA adjustment found one significant association. This finding was caused by a decreasing rate with dioxin in children of Ranch Hands, a finding opposite to the expected dose-response and most likely due to chance. Analyses of specific delays in development without pre-SEA adjustment found one significant association, caused by the rate being higher in children of Ranch Hands with low dioxin levels than in children of Comparisons. The rate in children of Ranch Hands with high dioxin levels was not significantly different from the rate in children of Comparisons.

These findings are weak, inconsistent and often opposite to the expected dose response. They are not supportive of a hypothesis of an adverse association between dioxin and delays in development or hyperkinetic syndrome.

Multiple Birth Defects

Of 1772 post-SEA children included in these analyses, 57 had multiple defects that could not be attributed to recognized syndromes. The few significant associations with dioxin were caused by increased rates of multiple birth defects in children of Ranch Hands with low dioxin levels relative to children of Comparisons. The rates in children of Ranch Hands with the highest dioxin levels were not significantly elevated. These findings are weak and inconsistent with the expected dose-response. We conclude that there is no evidence in these data that dioxin is adversely associated with multiple birth defects.

Infant and Neonatal Mortality

Analyses of infant death were either negative or could not be carried out due to insufficient data. Analyses of neonatal death found two significant associations, both caused by the rate of neonatal death being lower in children of Ranch Hands with the highest dioxin levels than in children of Comparisons (opposite to the expected dose response). We conclude that there is no association between dioxin and infant or neonatal mortality.

Summary

Extensive analyses of verified birth defects and other reproductive outcomes were conducted with the father's serum dioxin level as the measure of exposure.

The lack of significant association between dioxin and total conceptions and between dioxin and any considered semen characteristic provide no support for the claim that Ranch Hand dioxin exposure is adversely related to the ability to father children. Similarly, the lack of association between dioxin and miscarriage, total adverse outcome, birth weight, any of 13 categories of birth defects and neonatal death provides no support for the idea that dioxin is adversely related to reproductive outcomes in this population.

The few positive associations found between dioxin and reproductive outcomes were generally weak, inconsistent or biologically implausible. These data provide no support for the hypothesis that paternal dioxin exposure is adversely associated with reproductive outcomes. Whether dioxin exposure of the mother before or during pregnancy results in abnormalities in the developing fetus or child could not be addressed in this study and remains an open question.

1. INTRODUCTION

1.1 Background

The Air Force is conducting a 20-year prospective study of health, mortality and reproductive outcomes of members of Operation Ranch Hand, the unit responsible for aerial spraying of herbicides in Vietnam from 1961 to 1971. A comparison group of Air Force veterans who served in Southeast Asia (SEA) during the same period but who were primarily involved with air cargo missions and who were not occupationally exposed to herbicides and their contaminant 2,3,7,8 tetrachlorodibenzo-p-dioxin (dioxin) was selected. The study, called the Air Force Health Study (AFHS), is now in its tenth year.

At the baseline physical examination in 1982 [1] and again in 1985 [2] and 1987 [3,4,5], participants and their spouses or sexual partners were asked about the birth defect and mortality status of their children and occurrences of miscarriage, stillbirth and induced abortions. An initial analysis of birth defects as reported at the baseline examination [1] revealed a significant change in the relative risk of total reported birth defects with time. The Ranch Hand rate was lower than the Comparison rate among children born before the father's service in SEA but higher among children conceived after the father's service in SEA. This finding motivated complete medical record verification of the birth defect status and subsequent physical disability or mental impairment up to the age of 18 of all children fathered by the participants. Verification took place during 1985 through 1990. During the same period, chemists at the Centers for Disease Control (CDC) developed a serum assay for dioxin and demonstrated its suitability as a substitute for the more invasive assay of dioxin in adipose tissue [6,7]. In 1987, the CDC and Air Force collaborated in a pilot study of 200 AFHS participants to determine the feasibility of using the assay as a direct measure of dioxin exposure [8]; the results demonstrated that Ranch Hands have levels significantly higher than those of the Comparisons. Based on these data, the serum dioxin level is considered the best available measure of exposure to dioxin in occupationally exposed populations. Subsequently all willing participants in the 1987 physical examination were assayed. During 1989 and 1990, all physical health data arising from the 1987 physical examination were assessed for dioxin-related effects using serum dioxin levels as the measure of exposure. This report summarizes the analysis of verified reproductive outcomes fathered by Ranch Hands and Comparisons versus the dioxin body burden of these men.

1.2 Inclusion Criteria

All biologic conceptions and children of study participants for whom a quantifiable dioxin assay was completed by January 1990 were considered in these analyses. At that time, 932 Ranch Hands and 1202 Comparisons had serum specimens analyzed by the CDC. Of the 932 Ranch Hand specimens, 60 were reported by the CDC as not quantitable by the analytic method. Thus, the reproductive outcomes of 872 Ranch Hands were included in these analyses. Of the 872, 791 Ranch Hands fathered 2533 conceptions and 768 fathered 2074 live births.

Of the 1202 Comparisons assayed for dioxin, the reproductive outcomes of 166 were excluded from analysis because the dioxin results were reported by the CDC as not quantitable by the analytic method (n=142) or because the Comparison's dioxin level was greater than 10 parts per trillion (n=24). Therefore, the biologic reproductive outcomes of 1036 Comparisons were eligible for inclusion in these analyses. Of the 1036, 942 Comparisons fathered 2956 conceptions and 918 fathered 2440 live births. Throughout this report, live births are considered synonymous with children.

1.3 Statistical Methods

Models

The statistical analyses in this report are based on assumptions and models developed in 1988 after the publication of the Ranch Hand pilot study [7] and first dioxin half-life study [8]. At that time, available data regarding the elimination of dioxin in humans supported the following summary statements:

- Measurements following the ingestion of dioxin by an individual showed that dioxin elimination appeared to be by first order mechanisms [9].

- Air Force data on 36 Ranch Hand veterans with dioxin body burdens measured in blood drawn in 1982 and 1987 produced a median half-life estimate of 7.1 years [8]. The lack of correlation between individual half lives and current dioxin levels supported a first order elimination assumption.

- Assay results on 872 Ranch Hands and 1060 Comparisons, including the 24 with 10 or more parts per trillion (ppt), shows that the dioxin (D) concentrations are log-normally distributed with the Ranch Hand distribution significantly shifted to the right of the Comparison distribution. The Comparison median is 4.22 parts per trillion (ppt); the 98th percentile of the Comparison distribution is 10.38 ppt. The Ranch Hand median is 12.84 ppt and the 98th percentile is 166.43 ppt. Based on these data, levels at or below 10 ppt are considered background.

The term "elimination" denotes the overall removal of dioxin from the body. Some analyses in this report require the assumption that the amount of dioxin in the body (D) decays exponentially with time according to the model $D=I\exp(-rT)$, where I is the initial level, $r=\log 2/H$, H is the half-life, and T is the time between the end of the tour of duty in SEA and the dioxin blood draw in 1987. This exponential decay law is termed first order elimination.

The first order elimination assumption is equivalent to assuming a one compartment model for dioxin distribution within the body. While a multicompartment model incorporating body composition and dioxin binding to tissue receptors would provide a detailed description of dioxin concentrations in

different compartments, published multicompartment models for dioxin distribution within the body predict first order elimination of dioxin, overwhelmingly due to fecal excretion [10]. Direct assessment of the first-order assumption has not yet been carried out.

The term "current dioxin" refers to the serum lipid-weight concentration of dioxin, expressed in parts per trillion (ppt) [6,11]. The lipid-weight dioxin measurement is a derived quantity calculated from the formula ppt=ppq(102.6/W), where ppt is the lipid-weight concentration, ppq is the actual weight of dioxin in the serum sample in femtograms, 102.6 corrects for the average density of serum, and W is the total lipid weight of the sample.

The correlation between the serum lipid-weight concentration and adipose tissue lipid-weight concentration of dioxin has been observed to be 0.98 in 50 persons from Missouri [12]. Based the same data, the partitioning ratio of dioxin between adipose tissue and serum on a lipid weight basis has been estimated as 1.09 (95% CI: 0.97-1.21). These data suggest that there is a 1:1 partitioning of dioxin between serum lipid and adipose tissue. Measurements of dioxin in adipose tissue generally have been accepted as representing the body burden concentration of dioxin. The high correlation between serum dioxin levels and adipose tissue dioxin levels suggests that serum dioxin is also a valid measurement of dioxin body burden.

There are two limitations to the available data:

1) While Ranch Hand and ingestion data do not appear to contradict a first-order elimination assumption, no serially repeated dioxin assay results are yet available to evaluate directly the adequacy of the first order elimination model in humans.

2) At this time, it is not known whether Ranch Hands with dioxin burdens at or below 10 ppt were exposed and their body burdens have decayed to background levels since their tour of duty in Vietnam or whether they were not exposed at all during their tour in Vietnam.

Because first-order elimination is suggested, but not directly validated in humans, the dioxin versus reproductive outcome relationship in Ranch Hands was assessed using two models.

The two models are:

Model 1: $\text{logit}(p)=\beta_0+\beta_1\log_2(I)$

Model 2: $\text{logit}(p)=\beta_0+\beta_1\log_2(D)+\beta_2 T+\beta_3 T\log_2(D)$

where $\text{logit}(p)=\log(p/(1-p))$

p=probability of an adverse reproductive outcome
I=extrapolated initial dose assuming first order elimination, $I=D\exp[T\log(2)/H]$
T=time between the end of the Vietnam Ranch Hand tour of duty and the 1987 dioxin blood draw
D=current dioxin body burden determined in 1987
H=dioxin half-life in Ranch Hands assuming first order elimination (7.1 years)

Both models rely on the assumption that Ranch Hands received a single dioxin dose in Vietnam and only background exposure thereafter. This is a simplification of the process by which Ranch Hands accumulated dioxin during their tour of duty in Vietnam; however, the Ranch Hand tours generally were short, approximately 1 year, relative to the time elapsed since their tours. Hence, additional knowledge regarding the accumulation of dioxin during an individual Ranch Hand's tour, were it to become available would not change conclusions drawn from any of the statistical analyses presented in this report.

Because the initial dioxin level (I) is computed using the first order elimination law with a fixed half-life of 7.1 years, Model 1 is directly dependent upon the first order elimination assumption. This model further requires that the half-life is fixed at 7.1 years. Model 2 is an extension of Model 1. It depends on the equation $\log(I)=\log(D)+T\log(2)/H$, which follows from the first order elimination law. In Model 2, the quantity $\log(2)/H$ is not specified and is identified as β_2, the coefficient of T. Hence, Model 2 also relies on the first order elimination and constant half-life assumptions, but does not require specification of the half-life. All reproductive outcome data were analyzed with both models to reduce the likelihood that an effect would be missed due to incorrect specification of the half-life.

The introduction of the time-by-current dioxin interaction ($\beta_3 T\log(D)$) in Model 2 allows investigation of the dioxin versus reproductive outcome relationship with respect to time. For example, an effect would be detected by Model 2 if there was no relationship between reproductive outcome and dioxin among Ranch Hands whose time since tour is relatively short and a strong positive association among Ranch Hands whose time since tour is longer. In this case, if the effect were strong enough, the interaction coefficient (β_3) would be significantly different from zero. Analyses within time strata would find the coefficient β_1 of $\log_2(D)$ significantly different from zero and positive for large values of time (T); β_1 would not be found significantly different from 0 for small values of T. An effect of this kind might be due to the passage of time or to a higher initial dioxin level received by Ranch Hands in the later time stratum.

Because it is not known whether Ranch Hands with background levels (D≤10 ppt) of current dioxin (n=347) received a dose above background levels in Vietnam, all analyses based on Models 1 and 2 were carried out with these Ranch Hands excluded. Additionally, since 10 ppt may be considered arbitrary or too conservative, all analyses based on Models 1 and 2 were also carried out with Ranch Hands having less than or equal to 5 ppt (n=125) excluded. With the second approach, it is assumed that Ranch Hands currently having more than 5 ppt (the approximate Comparison median dioxin level) were exposed in Vietnam and those with less than 5 ppt were not. The numbers 5 and 10 correspond to the approximate median and 98th percentile of the Comparison current dioxin distribution.

The exclusion of Ranch Hands having background dioxin levels (D≤10 ppt) was imposed to address the unknown exposure history of this subgroup. There were 347 Ranch Hands in this "Unknown" category. Alternatively, only those with less than or equal to 5 ppt (n=125) were excluded. The intent of these two analyses was to "trap" the true dioxin versus reproductive outcome relationship between them. However, if the results of the D>5 analyses appear to be statistically significant more often than those of the D>10 analyses, this could be due to the larger sample sizes of the D>5 ppt cohort or it could be due to the uncertainty of true exposure Ranch Hands between 5 ppt and 10 ppt. There are no additional data available at this time with which to resolve these two interpretations.

Initial and current dioxin were analyzed in their continuous form, but trichotomized for tabular presentation. The time between the end of the tour of duty in SEA and the 1987 dioxin blood draw is dichotomized to 18.6 years (corresponding approximately to the year 1969), the approximate median time of service in SEA of Ranch Hands with more than 5 ppt. Ranch Hands with less than or equal to 18.6 years since duty in SEA are said to have "late" tours of duty. Ranch Hands with more than 18.6 years since duty in SEA are said to have "early" tours of duty. The cutpoints for stratifying initial and current dioxin levels were the approximate 25th and 75th percentiles and were specific to Ranch Hands with more than 5 ppt or to Ranch Hands with more than 10 ppt current dioxin.

We also assessed the reproductive consequences of current dioxin body burdens above background with a third model (Model 3) that required no assumptions about when or how increased dioxin body burdens were attained and was applied to both Ranch Hand and Comparison data. This model assessed reproductive outcomes versus current dioxin body burden (D) categorized in four levels. Table 1-2 defines the four categories of D.

Table 1-1

Dioxin Category Definitions for Model 3

Category	Definition
Background	Comparisons with up to 10 ppt current dioxin
Unknown	Ranch Hands with up to 10 ppt current dioxin
Low	Ranch Hands with more than 15 and up to 33.3 ppt current dioxin
High	Ranch Hands with more than 33.3 ppt current dioxin

The cutpoint between the Low and High categories (33.3 ppt) is the approximate median dioxin level of Ranch Hands having more than 15 ppt. Reproductive outcomes of Ranch Hands having between 10 ppt and 15 ppt were excluded from these categorized dioxin analyses in an attempt to avoid misclassification of the Unknown and Low categories.

The third model is given by $\text{logit}(p)=\beta_0+\beta_1 d_1+\beta_2 d_2+\beta_3 d_3$, where p=probability of an adverse reproductive outcome d_1, d_2 and d_3 are indicators for the Unknown, Low and High dioxin categories. The unadjusted Model 3 analysis first tests the hypothesis that $\beta_1=\beta_2=\beta_3=0$ and then individually tests the hypotheses that $\beta_1=0$, $\beta_2=0$ and $\beta_3=0$.

Covariates

When appropriate, analyses were adjusted for as many as 8 covariates, abbreviated and defined in Table 1-2.

Table 1-2

Candidate Covariates for Adjusted Analyses

Covariate	Abbreviation	Definition
Race of the father	RACE	Black or Nonblack
Mother's smoking history during pregnancy	SMOKE	Mother smoked (yes or no per pregnancy)
Mother's drinking history during pregnancy	DRINK	Mother drank (yes or no per pregnancy)
Mother's age	M-AGE	Mother's age at time of child's birth (outcome)
Father's age	F-AGE	Father's age at time of child's birth (outcome)
Time of conception relative to tour	C-TIME	Number of years the child was conceived after the father's last return from SEA
Father's military occupation in SEA	OCC	Officer, enlisted flyer, or enlisted ground personnel
Industrial chemical exposure	CHEM	Father's exposure (yes,no) to industrial chemicals

Post-SEA Analyses

We used Models 1, 2 and 3 to assess the significance of the association between reproductive outcome and paternal dioxin level in children conceived during or after the father's duty in Southeast Asia (SEA). Such children are referred to as "Post-SEA" children. Children conceived prior to the father's duty in SEA are called pre-SEA children. These post-SEA analyses are based upon the assumption that the fathers had equal opportunity for assignment to a Ranch Hand or Comparison unit in Southeast Asia and, therefore, that their reproductive histories prior to their tour of duty in SEA are not different. These analyses were carried out without and with adjustment for covariates.

Pre-Post SEA Analyses

Because the validity of the assumption of equal pre-SEA reproductive histories is unknown, analyses that take pre-SEA reproductive histories into account were also carried out. The goal of these analyses is to assess the significance of variation in the association between reproductive outcome and the father's dioxin level with the time of conception of the child relative to the father's duty in SEA. These analyses require no assumption about pre-SEA reproductive history but are more difficult to interpret. These analyses were not unadjusted for covariates because the presence of interactions would make interpretations even more difficult. The important aspects of these pre-post SEA analyses are

(a) the variation in the association between reproductive outcome and the father's dioxin level and
(b) the nature of the variation.

A hypothetical example of a dioxin effect using Model 3 would be an equal or higher prevalence of birth defects among pre-SEA Comparison children than among pre-SEA Ranch Hand children with the situation being reversed after the fathers duty in SEA. If a dose response was present among children of Ranch Hands conceived during or after the father's return from SEA, this would provide strong evidence of an adverse dioxin effect on birth defects.

Variation in the association between paternal dioxin and reproductive outcome with time of conception of the child relative to the father's SEA duty without a post-SEA dose-reponse are not interpretable as being related to the father's dioxin level because such variation could be caused by factors (such as maternal smoking) not under control in this study. Additionally, any association between pre-SEA birth defects and the father's dioxin level is a purely chance occurrence.

In a pre-post SEA analysis using Model 1, strong evidence for a dioxin effect would be revealed by no association between initial dioxin level and adverse reproductive outcome among pre-SEA children and an association among post-SEA children. If the association among post-SEA children was in the positive direction, these data would provide strong evidence for an adverse dioxin effect.

In a pre-post SEA analysis using Model 2, assessment of variation in association is multifactorial, the factors being (1) birth defect status of the child, (2) the father's current dioxin level, (3) time of conception of the child relative to the father's duty in SEA and (4) time since SEA duty. These analyses are necessarily complicated because this model employs current dioxin and time since duty in SEA separately rather than through a computed initial dose.

A hypothetical example of strong evidence for a dioxin effect using Model 2 is indicated if increased birth defects are seen only among children born within the first few years after exposure and not at all among children born

many years after exposure. If birth defects and current dioxin are unassociated among pre-SEA children and there is a dose response effect among post-SEA children whose father had late tours and no association among post-SEA children whose fathers had early tours, these data would provide strong evidence for a dioxin effect.

In Model 1, current dioxin is extrapolated to the initial dose in Vietnam rather than to the father's body burden at time of birth of his child. The time of conception of the child was a covariate. In analyses restricted to post-SEA children, this approach is nearly equivalent to one incorporating the extrapolation of the dioxin dose to the time of conception of the child. This approach has the added advantage that the same models are applicable in both the pre-post SEA and the post-SEA assessments.

Conventions

All analyses are displayed in tabular form and statistically interpreted. In those interpretations, all p-values are cited, regardless of their significance. All interactions are discussed and interaction tables are shown in the Appendix. The analyses and statistical interpretations constitute the bulk of this report and are provided to form a reference manual of data and results. Some tables are not analyzed because there is not sufficient data with which to confidently apply the statistical procedures that accompany each model. Rates are displayed in the tables for descriptive purposes; however, odds ratios were generally used in testing for significance. In adjusted analyses, significant covariates and interactions between covariates and dioxin are indicated under the heading "Covariate Remarks". The covariate remarks employ the covariate abbreviations shown in Table 1-2.

If the p-value for an interaction between dioxin and a covariate was greater than 0.01 and less than or equal to 0.05, the interaction was noted under covariate remarks and the analysis was rerun with the interaction removed from the model. The results are indicated with a triple asterisk (***). If the p-value for an interaction between dioxin and a covariate was less than or equal to 0.01, the interaction was noted under covariate remarks and no summary statistics are shown. In this case, summary statistics are replaced by four asterisks (****) and the interaction is displayed in the Appendix.

The reproductive outcomes considered in this report arise from conceptions fathered by the Ranch Hands and Comparisons who had a quantitable dioxin assay result. Birth defect status was verified on conceptions that resulted in a live birth. A conception is defined as any outcome of a fertilization. A live birth is a conception that produced a viable fetus. A viable fetus is a fetus, irrespective of its gestational age, that shows evidence of life (heart beats or respiration) at birth.

All conceptions, regardless of gestational period or outcome (induced abortion, miscarriage, stillbirth and live birth) reported by study participants or their spouses or partners were verified through the retrieval of medical documents and birth or death certificates. In the assessment of birth defects, developmental delays, and physical, mental and motor impairments, the providers of primary and hospital care were identified for each conception which resulted in a live birth. Each study participant and spouse or partner was interviewed by phone and a complete history was taken identifying each provider of care from the date of birth through the 18th year, or the death of the child. Where appropriate authorizations for the retrieval of medical records were obtained. All retrieved records were subjected to double review for the identification and classification of anomalies and morbid conditions identified in the records. All conditions were classified in accordance with the rules and conventions of the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) [13]. Additionally, all coded anomalies were reviewed by a CDC geneticist.

1.4 Sample Sizes

A total of 9,921 conceptions were reported by study participants, wives and partners. Every reported conception was subjected to verification as to whether or not a conception occurred and the outcome if a conception did occur. Of these, 9,891 (99.7%) were verified. Additionally, 953 relationships without conceptions were reported, of which 945 (99.2%) were verified.

All conceptions are summarized in Table 1-3 by verification status (verified, not verified) and father (study participant, not a study participant).

Table 1-3

Conceptions Categorized by Verification Status and the Father's Study Participation

Father's Study Participation	Verified (%)	Not Verified	Totals
Fathered by a Participant	8263 (99.7)	28	8291
Not Fathered by a Participant	1628 (99.9)	2	1630
Total	9891 (99.7)	30	9921

Verified conceptions and live births are summarized in Table 1-4 by restriction on the father (study participants and nonparticipants, study participants, study participants included in Models 1, 2 or 3).

Table 1-4

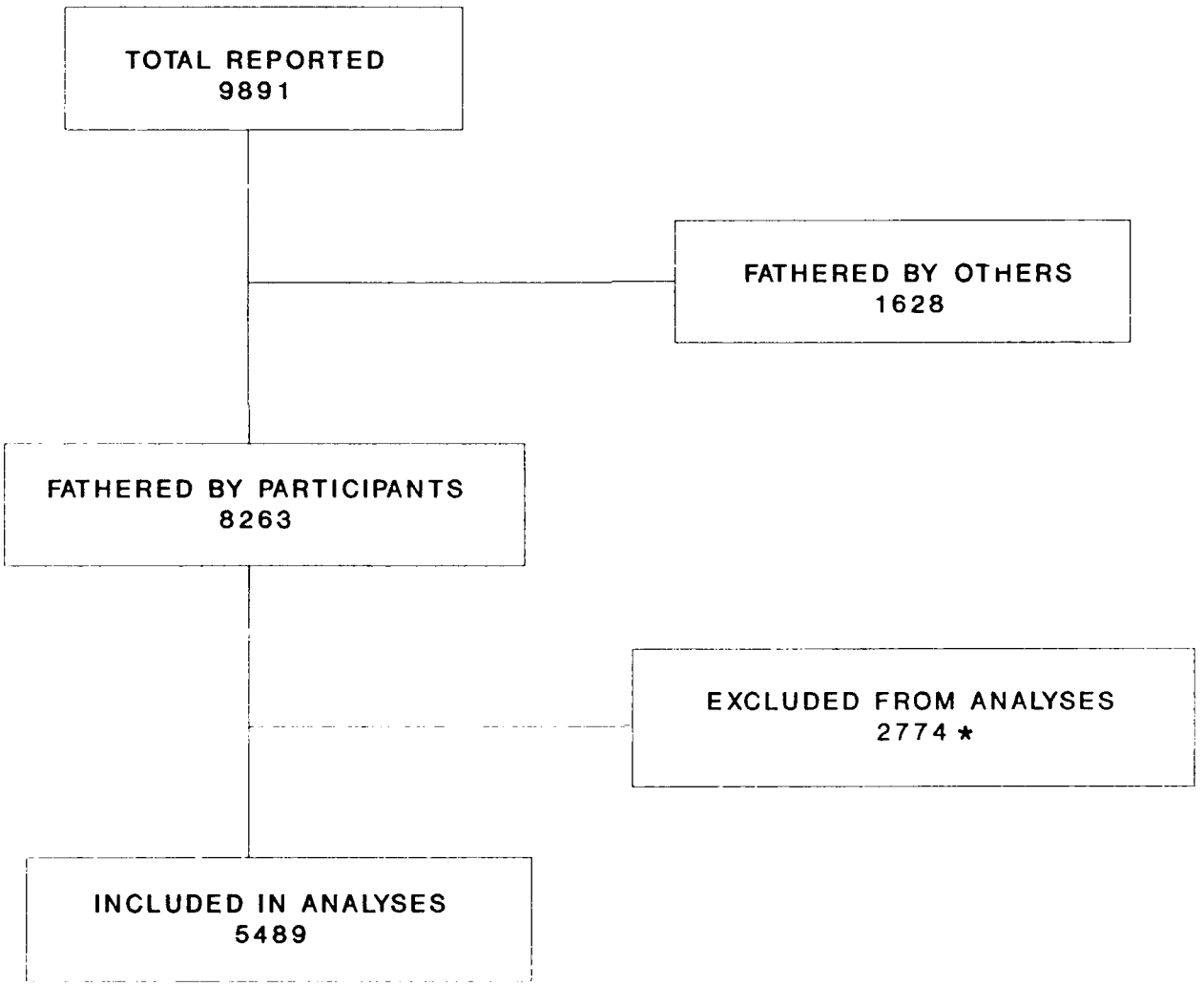
Overall Sample Sizes
Number of Conceptions, Live Births and Fathers

	Fathers								
	Study Participants and not Study Participants			Study Participants only			Study Participants included in Models 1, 2 or 3		
	RH	C	Total	RH	C	Total	RH	C	Total
Conceptions	4299	5592	9891	3506	4757	8263	2533	2956	5489
Fathers	1124	1588	2712	1098	1549	2647	791	942	1733
Live Births	3477	4613	8090	2850	3942	6792	2074	2440	4514
Fathers	1102	1562	2664	1062	1512	2574	768	918	1686

The sample sizes in Table 1-4 are graphically represented in Figures 1 and 2.

Figure 1

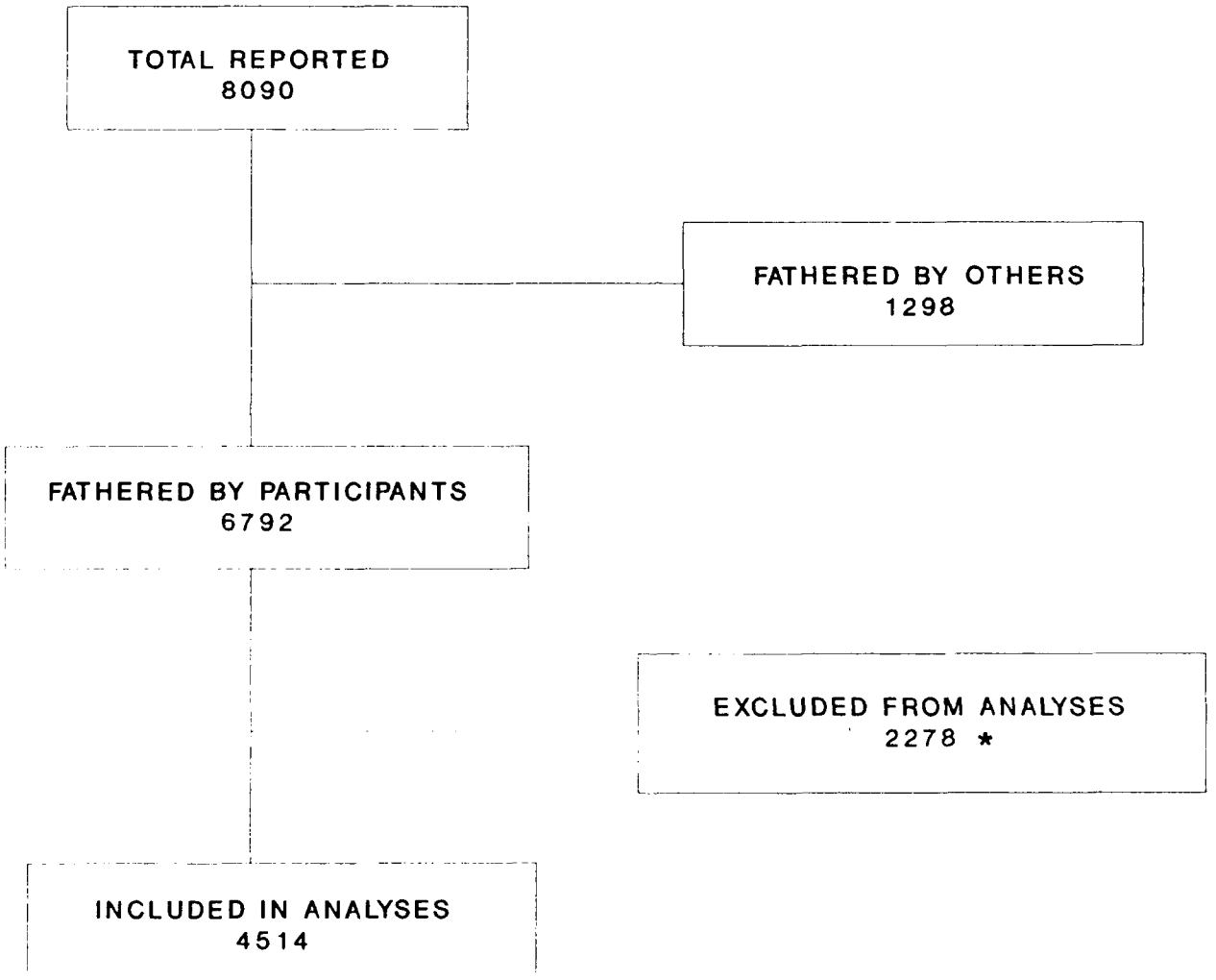
SAMPLE SIZES - CONCEPTIONS



* DIOXIN LEVELS UNAVAILABLE OR DID NOT SATISFY REQUIREMENTS OF THE MODELS

Figure 2

SAMPLE SIZES - LIVE BIRTHS



* DIOXIN LEVELS UNAVAILABLE OR DID NOT SATISFY REQUIREMENTS OF THE MODELS

All reproductive outcomes are summarized in Table 1-5 by the father's inclusion in the study, verification status and outcome.

Table 1-5

Reproductive Outcome versus Verification Status and Father's Study Participation

a) Fathered by a Participant

Outcome	Verified	(%)	Not Verified	Totals
Abortive Pregnancies				
Miscarriage	1124	(99.6)	4	1128
Induced Abortion	185	(92.5)	15	200
Tubal Pregnancy	44	(100)	0	44
Other	8	(100)	0	8
Births				
Live Birth	6792	(99.9)	9	6801
Stillbirth	110	(100)	0	110
Total	8263	(99.7)	28	8291

b) Not Fathered by a Participant

Outcome	Verified	(%)	Not Verified	Totals
Abortive Pregnancies				
Miscarriage	218	(99.5)	1	219
Induced Abortion	8i	(100)	0	81
Tubal Pregnancy	9	(100)	0	9
Other	0		0	0
Births				
Live Birth	1298	(99.9)	1	1299
Stillbirth	22	(100)	0	22
Total	1628	(99.9)	2	1630

Live births were also categorized as full siblings if all live births resulted from a participant impregnation of one woman, regardless of the number of relationships the participant had. Conceptions were categorized as full siblings if all conceptions resulted from a participant impregnation of one woman, regardless of the number of relationships the participant had.

All analyses were first carried out without restriction on sibship and again with restriction to full siblings. The restriction to full siblings was imposed to minimize genetic variability.

Table 1-6 shows the number of verified conceptions and live births fathered by the study participants included in this report, categorized by time of conception relative to SEA duty (pre-SEA, post-SEA), sibship (all conceptions, full siblings) and inclusion in any of the 3 statistical analysis according to the dioxin level restrictions of the models. For 131 outcomes gestation week was not available. In all but 9 of these outcomes (8 miscarriages and 1 induced abortion) the time relationship to the father's duty in SEA could be resolved.

Table 1-6

Counts of Verified Conceptions and Live Births Fathered by Participants Categorized by Time of Conception Relative to Tour of Duty, Sibship and Inclusion in the Analysis

a) Conception

Time of Conception Relative SEA Duty	Sibship Restriction	Analysis Inclusion	Count
Pre-SEA	All conceptions	Yes	3240
		No	1629
		Total	4869
	Full siblings	Yes	2774
		No	1412
		Total	4186
Post-SEA	All conceptions	Yes	2240
		No	1133
		Total	3373
	Full siblings	Yes	1765
		No	876
		Total	2641

Table 1-6 (Continued)

b) Live Births

Time of Conception Relative SEA Duty	Sibship Restriction	Analysis Inclusion	Count
Pre-SEA	All children	Yes	2742
		No	1403
		Total	4145
	Full siblings	Yes	2375
		No	1229
		Total	3604
Post-SEA	All children	Yes	1772
		No	875
		Total	2647
	Full siblings	Yes	1454
		No	737
		Total	2191

Live births not included in these analyses (Table 1-6) consists of children of Comparisons (n=1268) and Ranch Hands (n=733) whose dioxin assays were not available at the time of analysis, children of Ranch Hands (n=43) and Comparisons (n=166) whose dioxin result was available but not quantitable and children of Comparisons having more than 10 ppt current dioxin (n=68).

Of the 4514 live births included in the statistical analyses, 3829 (84.8%) were full siblings.

Table 1-7 categorizes all verified pre-SEA conceptions and live births by inclusion in each of the three statistical analyses. Table 1-8 shows the same categorization for full sibling live births. Corresponding summaries of post-SEA conceptions and live births are given in Tables 1-9 and 1-10.

Table 1-7

All Verified Pre-SEA Conceptions and Live Births by Inclusion in Each of the Three Statistical Analyses

		Analysis Model				
		Model 1	Model 2		Model 3	
			Time Since SEA (years)			
Outcome	Current Dioxin	Stratum	n	≤18.6 n	>18.6 n	Stratum	n
Conceptions	D>10 ppt	Low	293	162	113	Bkgd	1712
		Medium	406	218	207	Unk	691
		High	137	41	96	Med	344
						High	204
		Total	836	421	416		2951
	D>5 ppt	Low	352	194	174		
		Medium	726	374	326		
		High	194	79	125		
		Total	1272	647	625		
Live Births	D>10 ppt	Low	249	137	95	Bkgd	1459
		Medium	338	183	171	Unk	582
		High	113	37	78	Med	290
						High	168
		Total	700	357	344		2499
	D>5 ppt	Low	286	157	150		
		Medium	616	313	270		
		High	156	66	102		
		Total	1058	536	522		

Table 1-8

Verified Pre-SEA Full Sibling Conceptions and Live Births Fathered by Participants in Each of the Three Statistical Analyses

		Analysis Model				
		Model 1	Model 2		Model 3	
			Time Since SEA (years)			
Outcome	Current Dioxin	Stratum	n	≤18.6 n	>18.6 n	Stratum	n
Conceptions	D>10 ppt	Low	273	149	101	Bkgd	1450
		Medium	325	184	166	Unk	604
		High	121	39	81	Med	282
						High	176
		Total	719	372	348		2512
	D>5 ppt	Low	308	158	157		
		Medium	630	332	279		
		High	164	72	104		
		Total	1102	562	540		
Live Births	D>10 ppt	Low	231	124	85	Bkgd	1250
		Medium	276	155	143	Unk	514
		High	103	35	69	Med	244
						High	148
		Total	610	314	297		2156
	D>5 ppt	Low	252	126	145		
		Medium	545	276	237		
		High	135	60	88		
		Total	932	462	470		

Table 1-9

All Verified Post-SEA Conceptions and Live Births by the Fathered Inclusion in Each of the Three Statistical Analyses

		Analysis Model				
		Model 1	Model 2		Model 3	
			Time Since SEA (years)			
Outcome	Current Dioxin	Stratum	n	≤18.6 n	>18.6 n	Stratum	n
Conceptions	D>10 ppt	Low	136	76	59	Bkgd	1235
		Medium	310	161	146	Unk	367
		High	191	89	107	Med	212
						High	282
		Total	637	326	312		2096
	D>5 ppt	Low	199	118	77		
		Medium	392	211	183		
		High	280	136	146		
		Total	871	465	406		
Live Births	D>10 ppt	Low	106	62	40	Bkgd	981
		Medium	245	134	108	Unk	282
		High	157	72	93	Med	174
						High	227
		Total	508	268	241		1664
	D>5 ppt	Low	155	90	63		
		Medium	308	174	136		
		High	227	110	117		
		Total	690	374	316		

In Table 1-9, the total number of children entering the Model 3 statistical analysis (1664) is less than the total number (1772) of children entering any statistical analysis (Table 1-6) because the dioxin level and group membership requirements of the three models are not all the same.

Table 1-10

Verified Post-SEA Full Sibling Conceptions and Live Births Fathers by Participants in Each of the Three Statistical Analyses

		Analysis Model				
		Model 1	Model 2		Model 3	
			Time Since SEA (years)			
Outcome	Current Dioxin	Stratum	n	≤18.6 n	>18.6 n	Stratum	n
Conceptions	D>10 ppt	Low	98	56	42	Bkgd	982
		Medium	246	134	112	Unk	279
		High	159	75	85	Med	168
						High	232
		Total	503	265	239		1661
	D>5 ppt	Low	149	81	64		
		Medium	300	168	137		
		High	233	118	114		
		Total	682	367	315		
Live Births	D>10 ppt	Low	78	47	28	Bkgd	812
		Medium	206	115	92	Unk	221
		High	136	64	75	Med	148
						High	195
		Total	420	226	195		1376
	D>5 ppt	Low	114	59	53		
		Medium	245	144	106		
		High	198	98	97		
		Total	557	301	256		

For both pre-SEA and post-SEA live births the overall total for late and early tours (Model 2) is 1 more than the total for Model 1 in D>10 ppt. This occurred for all verified live births as well as full sibling live births. For one participant, the computed initial dioxin level was too low to be included in the low stratum for Model 1 while his current dioxin level did fall in the low stratum for Model 2. One of the two live births from this participant was conceived pre-SEA and the other post-SEA.

1.5 Birth Defect Category Definitions

Verified live births were categorized as either not defective or, if defective, to one or more of 13 categories defined by ICD-9-CM code [13]. Table 1-11 shows the ICD code definition and the number of verified defects among the verified 4145 pre-SEA and 2647 post-SEA live births. These are called CDC categories because they coincide with those used in the CDC birth defect study [14]. Rates are computed as the number of occurrences per 1000 live births.

Table 1-11

ICD Definition and Categorization of 1151 Birth Defects Among Verified Live Births Conceived Pre-SEA (n=4145) and Post-SEA (n=2647)

	Birth Defect Category	ICD-9 Definition	Pre-SEA n	Pre-SEA Rate	Post-SEA n	Post-SEA Rate
1.	Total congenital anomalies	740-759	438	105.7	518	195.7
2.	Nervous system anomalies	740-742	15	3.6	9	3.4
3.	Eye anomalies	743	16	3.9	20	7.6
4.	Ear, face and neck anomalies	744	18	4.3	28	10.6
5.	Circulatory system and heart anomalies	745-747	48	11.6	40	15.1
6.	Respiratory system anomalies	748	4	1.0	8	3.0
7.	Digestive system anomalies	749-751	43	10.4	52	19.6
8.	Genital anomalies	752	45	10.9	52	19.6
9.	Urinary system anomalies	753	47	11.3	34	12.8
10.	Musculoskeletal deformities	754-756	226	54.5	312	117.9
11.	Anomalies of the skin	757	38	9.2	62	23.4
12.	Chromosomal anomalies	758	6	1.4	11	4.2
13.	Other and unspecified anomalies	759	9	2.2	8	3.0

The total number of congenital defects is less than the sum of the numbers of defects in the 12 categories (2 through 13) because children with multiple defects are counted only once in the total congenital category but may be counted in more than one specific category.

All live births were categorized according to severity following the CDC definition [14]. Major defects were defined as those that potentially can affect survival, require substantial medical care, result in marked physical or psychological handicaps, or interfere with a child's prospects for a productive and fulfilling life. Minor defects were defined as those that are not associated with one or more of the above mentioned sequelae.

1.6 Statistical Power

The power of this study is limited by the sample sizes and birth defect rates observed in Comparison children. Because we verified almost 100% of the conceptions of Ranch Hands and Comparisons, these sample sizes are already maximized. One of the primary contrasts in these analyses is the Model 3 comparison of post-SEA birth defect rates in children of Ranch Hands in the High dioxin category (n=227) with children of Comparisons in the Background category (n=981). Table 5-7 summarizes birth defect rates in post-SEA Comparison children in each of the 13 CDC birth defect categories. The rates range from 2 per 1000 for other and unspecified anomalies to 204 per 1000 for total congenital anomalies. The power to detect a doubling of the birth defect rate (relative risk=2) in Ranch Hand children whose father is in the High dioxin category when the rate in Comparison children is 2 per 1000 is 3.5%. Thus, this study has virtually no power to detect a relative risk of 2 in birth defects having a prevalence of 2 per 1000. On the other hand, the power to detect a relative risk of 2 for total congenital anomalies (Comparison rate=204 per 1000) is 100%. Additionally, the power to detect a relative risk of 2 for musculoskeletal deformities (Comparison rate=132 per 1000) is also 100%. The power to detect a relative risk of 2 for birth defects having Comparison rates of 10, 20, 50, 80 and 90 per 1000 is 20%, 36%, 74%, 92% and 95%. Thus, this study has little power to detect relative risks of 2 in any category of reproductive outcome with a prevalence of 20 per 1000 or less and good power to detect relative risks of 2 for outcomes with prevalences of 80 per 1000 or more.

1.7 Reported versus Verified Reproductive Outcome

Because the baseline report presented only reported rather than verified reproductive outcome data, the correspondence between reported and verified data serves to link this report with the baseline analysis. Table 1-12 shows the correspondence between reported and verified reproductive outcome among the total of 8263 verified conceptions of Ranch Hand and Comparison fathers. Outcomes were reported by the mother or both father and mother. Most disagreements occurred in reported miscarriages. Thirty-five of 1199 reported miscarriages (2.9%) were verified as stillbirths.

Table 1-12

Correspondence between Reported and Verified Reproductive Outcome Among 8263 Conceptions

	Verified Outcome						
Reported Outcome	Induced Abortion	Live Birth	Other	Miscarriage	Still birth	Tubal Pregnancy	Total
Abortion	185	0	2	2	1	5	195
Live birth	0	6792	0	0	0	0	6792
Miscarriage	0	0	6	1120	35	38	1199
Stillbirth	0	0	0	2	74	1	77
Total	185	6792	8	1124	110	44	8263

In Table 1-13, all 6792 verified live births (see Table 1-6) are cross classified according to reported and verified birth defect. Reported defects were restricted to the CDC category "Total Congenital Anomalies." If the defect was reported by the mother or by both the father and the mother and within the Total Congenital Anomalies category, it was counted as a reported defect; otherwise, it was not counted. A child was also categorized as having a verified defect according to the CDC definition of "Total Congenital Anomalies." The results were stratified by the father's group membership (Ranch Hand, Comparison).

Table 1-13

Reported versus Verified Total Congenital Anomalies by the Father's Group Membership

Fathers Group	Reported Defect		Verified Defect Yes	(%)	No	(%)	Total
Ranch Hand	Yes		135	(4.7)	75	(2.6)	210
	No		278	(9.8)	2362	(82.9)	2640
		Total	413		2437		2850
Comparison	Yes		186	(4.7)	102	(2.6)	288
	No		357	(9.1)	3297	(83.6)	3654
		Total	543		3399		3942

Thus, 75 (2.6%) of 2850 Ranch Hand children were reported as defective and were verified as not being defective, representing over-reporting by Ranch Hand mothers. Similarly, 102 (2.6%) of 3942 Comparison children were over-reported by Comparison mothers. The percentages of children under-reported were also similar in the two groups (9.8% of Ranch Hand children and 9.1% of Comparison children). The association between reported and verified birth defects in Ranch Hand children does not differ significantly from that in Comparison children (p=0.738).

These data were further categorized according to the time of conception of the child relative to the father's duty in SEA. The results are shown in Table 1-14.

Table 1-14

Reported versus Verified Total Congenital Anomalies by Time of Conception Relative to SEA Duty and the Father's Group Membership*

Group	Time of Conception	Reported Defect		Verified Defect Yes	(%)	No	(%)	Total
Ranch Hand	Pre-SEA	Yes		57	(3.2)	40	(2.2)	97
		No		127	(7.0)	1581	(87.6)	1708
			Total	184		1621		1805
	Post-SEA	Yes		78	(7.5)	35	(3.4)	113
		No		151	(14.5)	780	(74.7)	931
			Total	229		815		1044
Comparison	Pre-SEA	Yes		97	(4.2)	62	(2.6)	159
		No		157	(6.7)	2024	(86.5)	2181
			Total	254		2086		2340
	Post-SEA	Yes		89	(5.6)	40	(2.5)	129
		No		200	(12.5)	1273	(79.5)	1473
			Total	289		1313		1602

*Based on 6791 live births because of missing gestation period for one Ranch Hand conception.

Table 1-14 shows that there is more under-reporting (12 to 14%) in post-SEA children than in pre-SEA children (approximately 7%) in both groups while over-reporting (approximately 2% in all strata) does not appear to be associated with either group or time of birth of the child. Analyses of these data found no variation in the association between reported and verified birth defects with group and time of conception ($p=0.282$), or with group after adjustment for time of conception ($p=0.821$).

1.8 Inclusion of Stillbirths

Stillbirths were not included among live births when assessing birth defects versus dioxin. A retrospective analysis of stillbirths suggests that the addition of stillbirths would not change the conclusions of this report. Therefore these data were not reanalyzed with stillbirths included in the birth defect analysis.

There were a total of 132 stillbirths of whom only 5 had verified defects (2 nervous system, 1 musculoskeletal, 2 other). Of the 132, 110 (Table 1-5) were fathered by a participant and were verified. Of the 110, 44 were conceived post-SEA. These 44 stillbirths had no verified birth defects. The remaining 66 stillbirths occurred before the father departed for SEA; 5 of these had verified birth defects.

The 44 stillbirths conceived during or after duty in SEA are distributed according to the father's dioxin body burden in Table 1-15.

Table 1-15

Distribution of the 44 Post-SEA Stillbirths by the Father's Dioxin Body Burden

Restriction	Dioxin Measure	Dioxin Category	Stillbirth count
D>10 ppt	Initial	Low	1
		Medium	6
		High	0
	Current	Low	3
		Medium	4
		High	0

Table 1-15 (Continued)

Restriction	Dioxin Measure	Dioxin Category	Stillbirth count
D>5 ppt	Initial	Low	5
		Medium	6
		High	2
	Current	Low	6
		Medium	4
		High	3
	Categorized dioxin	Background	13
		Unknown	7
		Low	1
		High	3

Because none of the 44 verified post-SEA stillbirths had defects, their inclusion in post-SEA birth defect analyses would increase the denominators of the post-SEA birth defect rates by the counts shown in Table 1-15. These changes in denominators would produce negligible changes in the results. Post-mortem examinations were performed on only 8 of the 132 stillbirths. Only 1 of the 8 had a verified birth defect (nervous system).

1.9 Correlation

A correlational analysis was conducted on 26 variables: current dioxin, 17 dependent variables (the 13 CDC birth defect categories, birth defect severity, birth weight, semen count, percent abnormal sperm) and the 8 covariates using the database of all verified live births (n=6792).

The 12 CDC birth defect categories (without total congenital anomalies) generally show correlations less than 0.25. The correlation between severity and the 12 CDC birth defect categories range from -0.16 (respiratory system anomalies) to -0.48 (musculoskeletal anomalies) while the correlation between severity and total congenital abnormalities is 0.851. The correlation between total congenital anomalies and musculoskeletal deformities was 0.725, indicating that most of the congenital anomalies were musculoskeletal deformities. Birth weight, sperm count and semen percent abnormal forms show correlations less than 0.10 with the 12 CDC birth defects categories, birth defect severity and total congenital anomalies.

The dependent variables were weakly correlated or not correlated with the covariates (all correlations were less than 0.10). The strongest correlation was between the father's military occupation in SEA and current dioxin (r=0.226). This is consistent with the dioxin levels within the occupational categories.

Among the covariates, the strongest correlations were among the mother's age, the father's age and the time of conception relative to duty in SEA (r=0.754). The correlation between the mother's age at the time of birth of the child and the time of conception relative to the father's return from SEA was 0.373. The correlation between the father's age at the time of birth of the child and the time of conception of the child relative to SEA duty was 0.505.

1.10 Interpretive Considerations

When interpreting the data in this report, careful consideration must be given to bias, interactions, consistency, multiple testing, trends, power limitations, strength of association, biologic plausibility, the evaluation of negative results and the coefficient of determination.

Bias

With the introduction of the dioxin assay as the measure of exposure, important sources of bias are reduced to violations of the underlying assumptions of the three statistical models upon which all analyses in this report are based.

Of the three models, Model 1 is the most vulnerable to bias, because it depends directly on two unvalidated assumptions: (a) that dioxin elimination is by first-order pharmacokinetics and (b) that all Ranch Hands have the same dioxin half-life. If dioxin elimination is first-order but some Ranch Hands have a shorter half-life than others, then there would be misclassification of initial dioxin exposure. If the reproductive outcome is not associated with a factor that affects the elimination rate, then estimates of the odds ratio for common outcomes associated with low and high levels of fathers dioxin will be biased toward unity. However, if the reproductive outcome is associated with a factor than affects the elimination rate, then the odds ratio will be biased away from unity.

Estimates of reproductive effects derived from Model 2 could be biased if some Ranch Hands were fast dioxin eliminators (have a short dioxin half-life) and some were slow eliminators (have a long half-life). If this phenomenon was associated with a covariate, lack of adjustment for this covariate would bias estimates of effect toward the null value. A similar concern arises regarding estimates of effect derived from Model 3. If, for example, a reproductive effect was expressed only many years after exposure, such an effect would probably only be apparent among children of Ranch Hands with the earliest tours of duty. The categorized current dioxin analyses were not adjusted for time since tour, however. Hence, it might not be possible to detect such an effect with that model because time since tour was not used for adjustment. For these reasons it is important to consider the results of analyses based on all three models.

Information bias, represented by over or under reporting of reproductive outcome, was precluded in this study by verifying all reproductive outcomes with birth certificates and medical records. It is possible, however, that Ranch Hand reproductive outcomes may be more verifiable because Ranch Hand children may have been taken to physicians more often than children of Comparisons in an effort, however subtle, by Ranch Hands to find defects in their children. This possibility is investigated in Chapter 11. This bias, if it does exist, would affect only estimates of effect derived from Model 3 because Comparison data were not used in Models 1 and 2. Information bias caused by errors in the data introduced through data entry or machine error is negligible because all data were completely verified after data entry and again before analysis.

Adjustments for Covariates and Interactions

The initial baseline reproductive outcome analyses [1], focused on overall group contrasts between conceptions and children of all Ranch Hands and Comparisons, which took advantage of the matched design. In those analyses, the matching variables age, race and military occupation were effectively eliminated as confounders. The present dioxin analyses of reproductive outcome do not benefit from the matched design because subjects in the categorized current dioxin analyses (Model 3) are not matched on date of birth, military rank, military occupation or race.

The adjusted models assessed the statistical significance of interactions between dioxin and the covariates to determine whether the relationship between the father's dioxin level and reproductive outcomes differed across levels of the covariate. In many cases the biological importance of significant interactions are unknown or uncertain. The biological relevance of a statistically significant interaction would be strengthened if the same interaction persisted in analyses of related reproductive outcomes. It is recognized that due to the large number of dioxin-by-covariate interactions that were examined for approximately 20 variables, some of the statistically significant dioxin-by-covariate interactions might be spurious (chance occurrences, not of biological or clinical relevance). This should be considered when interactions are interpreted.

Consistency

Ideally, a reproductive effect in children of Ranch Hands attributable to the father's herbicide or dioxin exposure would be revealed by internally and externally consistent findings. An internally consistent finding is one that does not contradict other data or findings in the same study. An externally consistent finding is one which has been established in other studies or one which does not contradict findings in other studies.

Assessment of external consistency is difficult in this study because prior information is weak or nonexistent. Internal consistency checks in this report are based on the following assumptions: (a) a genuine effect might be expressed in more than one birth defect category but not in all categories and (b) a genuine effect within a birth defect category would not likely be expressed in all subcategories.

Multiple Testing

Numerous dependent variables were considered because of the lack of a predefined reproductive endpoint. Each dependent variable was analyzed in many different ways to accommodate covariate information, different statistical models, and genetic variation. Even if a reproductive outcome is not related to dioxin level, about 5 percent of the many statistical tests in this report should be expected to be significant (p-value less than 0.05) by chance alone. Observing significant results due to multiple testing, even when there is no association between exposure and the outcome, is known as the multiple testing artifact and is common to all large studies. There is no statistical procedure available to distinguish between those statistical significant results that arise due to the multiple testing artifact and those that may be due to a bona fide effect. The authors have considered consistency, dose-response patterns, biologic plausibility and strength of association to weigh and interpret the findings.

Trends

Assessing consistent and meaningful trends is essential when interpreting any large study with multiple endpoints and covariates. However, caution must be used when assessing trends. Increased adverse reproductive outcomes with increased dioxin levels across related analyses might indicate a dioxin effect. In this case, it is important to note that there is moderate-to-strong correlation between such analyses. When variables are highly correlated, an effect on one would very likely be seen in the other. Hence, the strength of the trends as well as intercorrelations must be considered when assessing the suspected association.

Power Limitations

The fixed size of the Ranch Hand cohort limits the power of this study. This study has no power to detect low to moderate associations (relative risks less than 5) between the father's dioxin level and specific defects and syndromes which are so uncommon that few cases are expected among the Ranch Hand children in this study. This study has good power to detect relative risks of 2.0 or more with respect to outcomes, such as musculoskeletal deformities and total congenital anomalies, occurring at prevalences of at least 5

percent in unexposed populations. On the other hand, these sample sizes are sufficient to detect very small mean shifts with regard to birth weight, sperm count and the total number of conceptions. With regard to birth weight, this study has approximately 90 per cent power to detect a mean shift of 1 percent.

Strength of Association

Ideally, an adverse reproductive effect, if it exists, would be revealed by a strong association between dioxin and reproductive outcome. Statistically significant relative risks less than 2.0 are considered to be less important than larger risks because relative risks less than 2.0 can arise easily due to unperceived bias or confounding. Relative risks greater than 5.0 are less subject to this concern.

Biological Plausibility

Little or no information is available with which to hypothesize the "expected" pattern if dioxin were adversely related to reproductive outcome. Nevertheless, two patterns were considered as the "expected dose-response" if dioxin were adversely associated with reproductive outcome. These are (1) a positive linear association and (2) a nonlinear association in which the highest rates of anomaly occur at intermediate levels of paternal dioxin. The first appears plausible if dioxin is a teratogen. The second appears plausible if dioxin kills the embryo at high levels and is a teratogen at intermediate levels. Either of these hypotheses are subject to elimination from consideration if it is contradicted by the data. For example, the first would be dropped from consideration and the second would be supported if the number of conceptions is highest at intermediate dioxin levels. Conversely, the second would be dropped and the first supported if the number of conceptions is unassociated with dioxin or if the number of conceptions increases with dioxin.

Interpretation of Negative Results

A 1985 study [12] presents minimal sample-size criteria for proof of safety and hazard in studies of environmental and occupational exposures. The study was directed at rectifying widespread misconceptions about proof of safety often encountered in public health and safety issues. Thus, a lack of significant results relating dioxin to a particular effect only means that the study is unable to detect an association. This does not imply that an association not exist, but that, if it does exist, it was not detected.

Interpretation of the Coefficient of Determination

In a linear regression, the coefficient of determination, R^2, measures the proportionate reduction of the total variation in the dependent variable associated with the fitted model. However, a large value of R^2 does not necessarily imply that the fitted model is useful. Large values of R^2 would occur, for example, if the dependent variable is regressed on an independent variable with only two observed values. On the other hand, very small values of R^2 are generally seen in observational studies because little or no control has been applied in the assignment of values of the independent variables.

1.11 The Baseline Analysis

The primary focus of the baseline report [1] was the contrast of health and reported reproductive outcomes of Ranch Hands with Comparisons. Following the study protocol, Comparisons were labelled as Original or Replacement Comparisons. An Original Comparison was defined as a Comparison who was the first, by random selection from his matched set, to be invited to the baseline physical examination. If an Original Comparison refused to accept the invitation, another randomly selected Comparison from the same matched set was invited. Matched sets contained up to 10 Comparisons and had an average size of 6. Comparisons who accepted the invitation after Original Comparisons refused were called Replacement Comparisons.

There were 1045 Ranch Hands and 1224 Comparisons fully compliant to the 1982 (baseline) examination. Of the 1224 Comparisons, 773 were Original Comparisons. Study investigators emphasized Ranch Hand versus Original Comparison contrasts because they were concerned that scheduling delays may have biased the selection of Replacement Comparisons. Subsequent bias investigations in 1985 [2] suggested that the Replacement Comparisons were not a biased sample of Comparisons.

The baseline analysis [1] that prompted this expanded investigation was based on a cross classification of 4260 reported live births of Ranch Hands and Original Comparisons by reported birth defect (yes,no), group (Ranch Hand, Original Comparison) and time of birth relative to service in SEA (pre-SEA, post-SEA). A corresponding cross classification of 5242 reported live births of Ranch Hands and all Comparisons is shown in Appendix X, page AX-3, of the baseline report [1].

At baseline, data concerning fertility and reproductive events were collected during the questionnaire and physical examination. In addition to data collected from participants, questionnaires focusing on reproductive history were administered to all available spouses and partners. The data from the reconciliation of questionnaire responses constituted the database for statistical analysis. This reconciliation was based primarily on the mother's report and relied on the father's responses only when the mother's was not available. These baseline data were unverified and subjective. Additionally, when a child was reported as having multiple defects, only the most serious defect was analyzed.

The baseline tabulation is reproduced from the baseline report in Table 1-16. In Table 1-16, the birth defect rate is computed as the number of occurrences per 1000 live births.

Table 1-16

Baseline Counts of Reported Live Births by Reported Defect, Time of Birth Relative to the Father's Duty in SEA and the Father's Group Membership

Time of Conception	Group	Reported Birth Defect Yes	No	Total	Rate per 1000	Odds Ratio
Pre-SEA	Ranch Hand	78	1409	1487	52.4	0.815
	Original Comparison	80	1178	1258	63.6	
Post-SEA	Ranch Hand	76	757	833	91.2	1.456
	Original Comparison	44	638	682	64.5	

According to this classification, the Ranch Hand rate of reported birth defects (52.4 per 1000) was less than the Comparison rate (63.6 per 1000) among children born before the father's service in SEA. Among children born after the father's service in SEA, the Ranch Hand rate (91.2 per 1000) is higher than the Comparison rate (64.5 per 1000). The reversal of the odds ratio for reported birth defects from pre-SEA (OR=0.815) to post-SEA (OR=1.456) is statistically significant without adjustment for covariates (p=0.02) and after adjustment for the mother's age at the birth of the child, the mother's smoking during pregnancy, the mother's drinking during pregnancy and the father's age at the time of birth (p=0.04).

Reanalyses using fully verified data (see Chapter 5) confirm the results of the baseline analysis. Additionally, these new data demonstrate that the effects of over and under-reporting were negligible (see Section 1.7).

12. CONCLUSION

12.1 Introduction

Fear of cancer in Vietnam veterans and the occurrence of birth defects in their children has driven interest in the Agent Orange issue in veterans, the general public and federal and state legislatures. The Air Force began planning for the Air Force Health Study (AFHS) in late 1978 to evaluate these and other health issues in the group of Air Force veterans who handled and sprayed dioxin-containing herbicides on a daily basis in Southeast Asia (SEA) from 1962 to 1970. Initial physical examinations and questionnaires were performed in 1982, with subsequent evaluations in 1985, 1987 and currently in 1992.

Reproductive outcomes were assessed in the baseline AFHS report published in February 1984. The analyses of reproductive outcomes contained in that report were based on defects reported by the mothers of the children. No verification of those reports were carried out because the necessary medical records were not available at that time. Action was begun in 1985 to locate and obtain records for each conception, regardless of the mother's report. This task involved the collection of medical records on 9,921 conceptions and 8,100 live births.

12.2 Previous Studies

The scientific investigation of the effects of dioxin on the reproductive system has focused primarily on studies of exposed pregnant rodents and their offspring. Studies have identified a range of teratogenic abnormalities in fetuses when the mothers were fed varying amounts of dioxin, but few studies have been done following exposures of the fathers. Only a few studies evaluated the mating behaviors and reproductive success of male rats after exposure to dioxin at levels causing systemic toxicity. Mating behavior, litter size and birth defect rates were not affected by the father's exposure in one study [16], but the mating index decreased, sterility increased and the pregnancy index was normal in a second study [17]. Because of interspecies variability, the applicability of these animal studies directly to humans is in doubt.

All of the studies of reproductive effects of dioxin in humans conducted to date have relied on broad assumptions concerning the degree of dioxin exposure rather than upon direct measurement. Many studies have been no more than case reports of birth defects without any attempt to verify actual dioxin exposure. The birth defect studies conducted by the Government of Australia [18] and by the Centers for Disease Control [15] were unable to classify Vietnam veterans by their actual dioxin levels but only evaluated differences between Vietnam and non-Vietnam veterans, using presence in Vietnam as a surrogate for dioxin exposure. The studies of miscarriage in residents of Alsea, Oregon in 1978 were also unable to determine actual dioxin levels of

individual subjects. In all of these studies, the possibility of exposure misclassification limits the reliability of the results. Some previous studies were limited by small samples as well as lack of direct exposure measurements [19,20]. For example, studies of birth defects subsequent to a 1976 industrial accident in Seveso, Italy found no increased risk of major birth defects in the offspring of dioxin-exposed mothers, but the number of children of mothers with the highest likelihood of exposure was too small to assess specific categories of defects [21].

Recently, researchers have concentrated on the direct effects of dioxin in cultures derived from animal fetal tissue [22-25]. These studies have identified dioxin effects in neurological, palate, and kidney tissues. As in other animal studies, the applicability of these results to paternal human exposure remains debatable.

The study described in this report is the first to combine an accurate direct measurement of paternal dioxin level with documented and verified reproductive outcomes in a population of sufficient size to provide a reasonable opportunity to detect associations between paternal dioxin levels and a range of common reproductive outcomes. This study has good statistical power to detect relative risks of 2 for common birth defects such as musculoskeletal deformities and but no statistical power for relative risks of this order for rare conditions such as chromosomal abnormality or infant death.

The reduction of exposure misclassification and elimination of errors in determining the presence of birth defects has minimized the two most important sources of bias in epidemiologic studies of reproductive outcome. This study evaluates a range of reproductive outcomes including semen abnormalities, inability to conceive, prematurity (birth weight below 2500 grams), birth defects, neonatal and infant death and developmental abnormalities. This is the most comprehensive evaluation of paternal dioxin exposure and reproductive outcomes and, the most thorough accounting of the reproductive health of 1,686 men and the health and development to age 18 of their 4,514 children ever done.

12.3 Statistical Methods and Interpretation

The significance of the association between paternal dioxin level and post-SEA birth defects was assessed, in 12 separate series of analyses, within each of 13 categories of anomalies: total congenital, nervous system, eye, ear face and neck, circulatory system and heart, respiratory system, digestive system, genital, urinary, musculoskeletal, skin, chromosomal and other unspecified defects. Analyses were first conducted on all children and then, to minimize genetic variation, with restriction to full siblings. Within each of these two series, each analysis was carried out first without and then, when possible, with adjustment for covariates. A separate series of analyses, termed pre-post SEA, assessed the dioxin versus outcome after the father's service in Southeast Asia (post-SEA) with adjustment for outcomes which occurred before the father's service in Southeast Asia (pre-SEA).

Three dioxin measures were used: the extrapolated initial dose (Model 1), current dioxin with adjustment for time since departure from SEA (Model 2), and categorized current dioxin (Model 3). The first two of these models were applied only to children of Ranch Hands. The third included children of both Ranch Hands and Comparisons.

Assessment of external consistency is difficult in this study because prior information is weak or nonexistent. Internal consistency checks in this report were based on the following assumptions: (1) a genuine effect might be expressed in more than one birth defect category but not in all birth defect categories and (2) a genuine effect within a birth defect category would not likely be expressed in all subcategories.

Little or no information is available with which to hypothesize the "expected" pattern if dioxin were adversely related to reproductive outcome. Nevertheless, two patterns were considered as the "expected dose-response" if dioxin were adversely associated with reproductive outcome. These were (1) a positive linear association and (2) a nonlinear association in which the highest rates of anomaly occur at intermediate levels of paternal dioxin. The first appears plausible if dioxin is a teratogen. The second appears plausible if dioxin kills the embryo at the highest levels received by Ranch Hands and is a teratogen at intermediate levels. Either of these hypotheses were subject to elimination from consideration in the interpretations if it was contradicted by the data. For example, the first would be dropped from consideration and the second would be supported if the number conceptions were highest at intermediate dioxin levels. Conversely, the second would be dropped and the first supported if the number of conceptions increased with dioxin.

12.4 The Baseline Analysis

In the baseline AFHS report, the significance of the association between the father's group (Ranch Hand, Comparison) and the mothers report of any post-SEA birth defect was assessed with adjustment for reported pre-SEA defects. The analysis found significant variation in relative risk with time of birth relative to SEA. The Ranch Hand rate of reported pre-SEA defects was less than the Comparison rate and the Ranch Hand rate of reported post-SEA defects was greater than the Comparison rate. A repetition of this analysis with medical record verification replacing the mother's report found a similar significant variation in relative risk with time of birth relative to SEA.

Because the databases have been subjected to continual quality control since baseline, the baseline analysis was repeated, again using the mother's report. This repetition also revealed significant variation in relative risk with time of birth relative to SEA. Among pre-SEA children the rate of reported defects in Ranch Hand children (58.8 per 1000) was less than the Comparison rate (76.7 per 1000), but among post-SEA children the Ranch Hand rate (128.0 per 1000) was greater than the Comparison rate (86.2 per 1000).

The baseline finding motivated the verification of conception outcomes and birth defects which are the subject of this report. In the interim, advances in chemistry allowed the direct measurement of dioxin in human serum possible. Since 1987, 932 Ranch Hands and 1202 Comparisons have received the serum dioxin assay, providing a more direct assessment of dioxin exposure than simply group membership.

12.5 Semen

The statistical significance of the association between the father's dioxin level and sperm count and the percentage of abnormal sperm was assessed based on the testing of semen specimens collected during the baseline examination in 1982.

We found no significant association between dioxin and sperm count, low sperm count rate or the percentage of abnormal sperm. We conclude that there is no association between dioxin and any of these variables.

12.6 Conceptions

These analyses addressed the significance of associations between dioxin and miscarriage, total adverse outcome and total conceptions. Total adverse outcome was defined as miscarriage, tubal pregnancy, other (non-induced) abortive pregnancies, or stillbirth.

A pre-post SEA Model 3 analysis without restriction to full siblings found a significant association between categorized dioxin and total conceptions, caused by increased post-SEA conceptions in Ranch Hands in the High category (mean=2.47) relative to Comparisons in the Background category (mean=2.17). Significant associations in Model 1 analyses in full siblings were due to increasing mean numbers of post-SEA conceptions with dioxin. Significant associations in Model 2 analyses between dioxin and total conceptions among full sibling children were due to increasing conceptions with dioxin in Ranch Hands with late tours, opposite to the corresponding decreasing pre-SEA trends. These findings do not support and sometimes contradict the hypothesis that high levels of dioxin kill the embryo. Thus, these data do not support the theory that high anomaly rates should occur at intermediate levels of dioxin (this is the second of the two considered dose-response patterns). The "expected dose-response pattern" therefore is the linear one in which the highest anomaly rate occurs at the highest levels of dioxin.

Analyses of post-SEA miscarriage, total adverse outcome and total conceptions, were predominently negative. An unadjusted Model 2 analysis found significant variation in the association between dioxin and miscarriage with time since tour in Ranch Hands with more than 10 ppt current dioxin. This finding was caused by a positive association in conceptions of Ranch Hands with late tours and a negative association in Ranch Hands with early tours. That dioxin should act adversely in one stratum of Ranch Hands and beneficially in another is difficult to conceptualize and, therefore, this finding appears unrelated to dioxin.

Adjusted analyses of post-SEA miscarriage were either negative or were complicated by significant interactions with covariates. There was no pattern common to the 16 interactions and most were not consistent with the expected dose-response pattern. For example, an adjusted Model 2 analysis found a significant interaction with the father's military occupation in SEA. Among officers, miscarriages decrease with dioxin and among enlisted personnel there were no consistent patterns. In some enlisted strata the highest miscarriage rate occurred in Ranch Hands having intermediate dioxin levels and in one stratum Ranch Hands with the lowest dioxin level have the highest rate. An adjusted Model 3 analysis found a significant interaction with the mother's age. In mothers aged 27 or younger, Ranch Hands in the High category had a higher miscarriage rate (175.4 per 1000) than Comparisons in the background category (126.0 per 1000), but among mothers older than 27 the rate in the High category (126.6 per 1000) was less than the rate in the Background category (147.1 per 1000). In summary, the post-SEA miscarriage findings were either nonsignificant or, if significant, were inconsistent with the expected dose-response pattern or were complicated by covariate interactions that revealed no patterns suggestive of a dioxin effect. These findings are therefore most likely not related to dioxin.

Post-SEA analyses of total adverse outcome were either negative or found significant associations caused by trends that lack coherent explanation or were complicated by interactions with covariates. For example adjusted Model 1 analyses found significant interactions with the mother's age in children of Ranch Hands with more than 10 ppt and with the father's race and military occupation. The interaction with the mother's age was due to a positive association with dioxin in mothers aged 27 or younger and no association in mothers older than 27. The interactions with race and military occupation were caused by a negative association in children of nonblack enlisted groundcrew, a positive association in children of Black enlisted groundcrew and no association in children of officers. These results were generally weak, inconsistent and were sometimes contradictory or opposite to the expected dose response pattern. They are therefore most likely not related to dioxin.

Post-SEA analyses of total conceptions were either negative, inconsistent, or found positive associations between total conceptions and dioxin. For example, an adjusted Model 2 analysis found a significant interaction effect with time since tour caused by a positive association in children of Ranch Hands with late tours and a negative association in children of Ranch

Hands with early tours, but neither of these were significant. Two unadjusted Model 3 analyses found significant results caused Ranch Hands in the High dioxin category having more conceptions than Comparisons in the Background category. These findings contradict the theory that high levels of dioxin kill the embryo and are not indicative of an adverse effect of dioxin on total conceptions.

In summary, we find no evidence that dioxin is adversely associated with miscarriage, total adverse outcome or total conceptions. The observed increases in total conceptions with dioxin contradict and therefore eliminate from consideration the theory that dioxin at high levels kills the embryo. Therefore, the "expected dose-response" was reduced to a single pattern: increasing anomalies with increasing paternal dioxin.

12.7 Birth Weight

Pre-post SEA analyses of birth weight were predominantly negative. The few significant findings were not suggestive of a dioxin effect. For example, a Model 1 analysis of birth weight found a significant interaction with time since tour caused by a decreasing birth weights in pre-SEA children and a increasing in birth weights in post-SEA children. This change was due to an increase mean birth weight from pre- to post-SEA in children of Ranch Hands with the highest dioxin levels. Because low birth weights were considered adverse, this finding is not interpretable as an adverse effect of dioxin. A similar significant interaction was found after restriction to full siblings. In a Model 3 analysis of low birth weight, a significant interaction was found with time of conception in the contrast of children of Ranch Hands in the Unknown category with children of Comparisons in the Background category. Among pre-SEA children, the rate of low birth weight in Ranch Hand children (61.2 per 1000) was less than that in children of Comparisons (73.5 per 1000) and in post-SEA children, the rate in Ranch Hand children (93.3 per 1000) was greater than that in children of Comparisons (41.9 per 1000), but this change was due more to the decrease in the Comparison rate than to the increase in the Ranch Hand rate, a pattern that cannot be attributed to dioxin. A similar finding was revealed after restriction to full sibling children.

Post-SEA analyses of birth weight were generally negative or were complicated by interactions with covariates that made no sense. For example, a Model 1 analysis of birth weight found significant interaction with the father's race and the mothers' smoking, due to a significant decrease in birth weight with dioxin in children of Black fathers whose mother did not smoke during pregnancy and a borderline significant weight reduction in children of nonblack fathers whose mother did smoke during pregnancy. After restriction to full siblings, significant interaction with only the mother's smoking was found. In that analysis, there was a significant reduction in birth weight with dioxin in children of mothers who smoked during pregnancy and no significant reduction in children of mothers who did not smoke during pregnancy. A Model 2 analysis of birth weight found a significant interaction with the father's race; birth weight decreased borderline significantly with dioxin in children of Black fathers who had early tours but there were no

significant associations in the other 3 strata. After restriction to full siblings, no significant interactions with covariates were found, but in that analysis, birth weight decreased with dioxin in children of fathers who had late tours and increased in children of father's who had early tours but neither of these associations was significant. A Model 3 analysis of birth weight found a significant interaction with the father's race; the birth weight of children born to Black Ranch Hands in the High category was significantly less than of children born to Black Comparisons in the Background category and a weaker reduction was found in children of nonblack Ranch Hands in the High category. After restriction to full siblings, children of Ranch Hands in the High category were found to have significantly lower birth weight than children of Comparisons in the Background category.

Post-SEA analyses of low birth weight were generally negative or were complicated by interactions with covariates. For example, a Model 1 analysis found a significant interaction with the father's race and with the mother's drinking during pregnancy. This interaction was due to a significant increase in the rate of low birth weight with dioxin in children of nonblack father's whose mothers drank during pregnancy, however, the number of children (35) and the number with low birth weight (3) in this stratum were small; no significant associations were found in the other 3 strata. After restriction to full siblings, significant interaction was found with the mother's smoking during pregnancy and with the time of conception. This interaction was caused by a significant reduction in the risk of low birth weight with dioxin in children conceived within 2 years of the father's departure from SEA whose mother did not smoke during pregnancy. A Model 2 analysis of low birth weight found a significant interaction with time of conception. This interaction was caused by a significant increase in risk with dioxin in children conceived more than 6.5 years after the father's departure from SEA whose father had an early tour (relative risk=1.84). In the same analysis, however, there was a borderline significant decrease in risk with dioxin in children conceived within 2 years of the father's departure from SEA whose father had an early tour (relative risk=0.40). After restriction to full siblings, no significant associations were found between low birth weight and dioxin. A Model 3 analysis of low birth weight found a significant interaction with the father's military occupation in SEA, caused by a significant increase in risk with dioxin in children of fathers who were enlisted ground personnel (relative risk=2.58). After restriction to full siblings, significant a interaction with the father's race and military occupation in SEA were found, caused by significant increases in risk with dioxin in children of Ranch Hands in the High category who nonblack enlisted flyers or who were nonblack enlisted ground personnel.

These findings are generally weak and inconsistent. That (1) the nature of the interaction changes after restriction to full siblings and (2) birth weight decreases with dioxin in some strata and increases in others and (3) many of the interactions are based on sparse data, suggest that these findings are chance occurrences. We find no evidence in these data that birth weight is adversely associated with the father's dioxin level.

12.8 Pre-post SEA Birth Defects

Analyses were carried out to determine whether the variation in association between paternal dioxin level and birth defects with time of conception relative to SEA was attributable to a specific category of defect. After comprehensive reanalysis by birth defect category, the finding could not be attributed to a specific type of defect. Additionally, adjustment for the father's dioxin level revealed no significant results. Hence, the group difference found out baseline remains after verification but is not significantly associated with dioxin and cannot be attributed to a particular category of anomaly.

Each category of anomaly was additionally assessed for post-SEA dioxin effects with adjustment for pre-SEA anomalies using Models 1, 2 and 3. Each analysis was carried out first on all children and then with restriction to full siblings. All of these pre-post-SEA were unadjusted for covariates.

The only significant results in these analyses were the Model 2 assessments of digestive system anomalies and musculoskeletal deformities in children of Ranch Hands having more than 10 ppt current dioxin. The digestive system findings were caused by increasing post-SEA rates in children of Ranch Hands with late tours and no trends in post-SEA rates in children of Ranch Hands with early tours. The musculoskeletal findings were caused by decreasing post-SEA rates in children of Ranch Hands with early tours, opposite to a corresponding increasing trend in pre-SEA rates. These findings are inconsistent with each other and lack credible biologic explanation and therefore appear unrelated to dioxin.

In summary, a repetition of the baseline analysis with verified birth defect data revealed a significant change in birth defect rates with the Ranch Hand rate being less than the Comparison rate among pre-SEA children and greater than the Comparison rate among post-SEA children. However, after accounting for paternal dioxin level, we found no evidence that this effect was confined to a specific birth defect category and we found no significant association between this change in risk and dioxin. We conclude that this change in relative risk is unrelated to dioxin.

12.9 Post-SEA Birth Defects

The significance of the association between dioxin and post-SEA birth defects was assessed within each of 13 birth defect categories in four separates series of 3 analyses based on Models 1, 2 and 3, without adjustment for pre-SEA reproductive experiences. Analyses were first carried out on all children and then with restriction to full siblings and each of these was done first without and then with adjustment for covariates.

Few significant associations were found. Those that were found did not appear consistently across related analyses and most were not suggestive of a plausible dioxin effect. For example, a significant association was found between initial dioxin and circulatory system and heart anomalies, but the rate (6.4 per 1000) among children of Ranch Hands having the highest initial dioxin levels was less than that among children of Ranch Hands at the lowest dioxin levels (28.3 per 1000). In a Model 3 analysis restricted to full sibling children, a significant association was found between categorized dioxin and circulatory system and heart anomalies, but this was due to a high rate (47.3 per 1000) among children of Ranch Hands in the Low category relative to children of Comparisons in the Background category (17.2 per 1000) and a low rate among children of Ranch Hands in the High category (0 per 1000). In a Model 2 analysis, a significant association was found between current dioxin and anomalies of the ear, face and neck, but this was caused by an increase in anomalies with dioxin among children of Ranch Hands having early tours and a decrease in anomalies among children of Ranch Hands having late tours. A significant association was found in a Model 3 analysis of genital anomalies, but the pattern was not consistent with the expected dose-response. The rate among children of Ranch Hands in the Low dioxin category (51.7 per 1000) was greater than that among children of Ranch Hands in the High category (13.2 per 1000).

Several adjusted analyses of post-SEA total congenital anomalies and musculoskeletal deformities were complicated by significant interaction with covariates. Examination of these did not reveal meaningful patterns. For example, Model 3 analyses of total congenital anomalies found a significant interaction with the father's military occupation in SEA. Among officers, children of Ranch Hands in the High dioxin category had a lower rate (0 per 1000) than children of Comparisons in the Background category (217.9 per 1000). Among children of flying enlisted and enlisted ground personnel, children of Ranch Hands in the Low dioxin category had higher rates (433.3 per 1000 and 317.3 per 1000) than children of Comparisons in the Background category (228.9 per 1000 and 212.7 per 1000) but the rates in children of Ranch Hands in the High category were not significantly elevated. A Model 3 analysis of musculoskeletal deformities also found a significant interaction with the father's military occupation in SEA. In a pattern similar to that of total congenital anomalies, children of Ranch Hand officers in the Low category had a low rate (0 per 1000) relative to children of Comparisons in the Background category (155.6 per 1000) and the rates in children of enlisted flyers and enlisted ground personnel were not significantly elevated with respect to the rate in corresponding children of Comparisons in the Background category. In summary, analyses of total congenital anomalies and musculoskeletal deformities found significant variation in relative risk, but no clear pattern emerged. This suggests that these results are chance occurrences and that there is no underlying association with dioxin.

In conclusion, there is no consistent evidence of an association between dioxin and birth defects among post-SEA children. These findings are weak, inconsistent and lack credible biologic interpretation. They therefore appear unrelated to dioxin.

12.10 Birth Defect Severity

All live births were assigned to one of three birth defect severity categories (major, minor, none). The significance of the association between dioxin and birth defect severity was assessed with Models 1, 2 and 3 under various combinations of constraints on severity, sibship and statistical adjustment.

Unadjusted pre-post SEA analyses were carried out with severity reduced to 2 categories (major, not major), first based on all children and then with restriction to full siblings using each of the 3 models.

Pre-post SEA analyses of birth defect severity in two categories found significant associations in Model 3 but not in Model 1 or Model 2 analyses. The Model 3 findings were caused by the rate of major post-SEA defects being elevated in Ranch Hands in the Low dioxin category relative to Comparisons in the Background category. In the analysis of all children, the Low and Background rates were 126.4 and 57.1 per 1000; in full siblings the rates were 121.6 and 56.7 per 1000. However, the rate of major defect was not significantly elevated in children of Ranch Hands in the High dioxin category. The post-SEA rate of all children of Ranch Hands in the High category was 57.3 per 1000 and the rate of full siblings of Ranch Hands in this category was 46.2 per 1000. These results appear inconsistent and therefore artifactual. We conclude that there is no consistent evidence that post-SEA birth defect severity is associated with dioxin after adjustment for pre-SEA severity.

Model 2 analyses of post-SEA severity in 3 categories found significant associations with dioxin in all children and in full siblings, but these findings were caused by the highest rate of major defect occurring in children of Ranch Hands having early tours and intermediate dioxin levels. Corresponding analyses of severity in 2 categories were negative. These findings appear inconsistent with the expected patterns and are not suggestive of a dioxin effect.

Without and with restriction to full siblings, unadjusted Model 3 analyses of severity in 3 and in 2 categories found significant differences between children of Ranch Hands in the Low dioxin category and children of Comparisons in the Background dioxin category. These findings were due to a high rate of major defects in children of fathers in the Low dioxin category relative to children of fathers in the Background category, while the rate of major defects in children of fathers in the High dioxin category is not significantly elevated. These patterns are consistent with those of the Model 2 analyses but are not suggestive of a dioxin effect.

An adjusted Model 3 analysis of severity in 2 categories found significant variation in effect with the mother's smoking and the father's military occupation in SEA. However, within 2 of the 4 smoking by occupation strata the children of fathers in the High dioxin category had the lowest rate of major defects. Significant findings in 1 of these strata were caused by the rate of major defect in children of Ranch Hands in the Low dioxin category being greater than that of children of Comparisons in the Background category.

In summary, analyses of birth defect severity found few significant associations. Those associations that were significant were generally caused by children of Ranch Hands with intermediate dioxin levels having the highest rates of major birth defects. In some of these analyses children of Ranch Hands with the highest dioxin levels had the lowest rates of major defects. These patterns are inconsistent with the expected dose-response pattern and are therefore not suggestive of a dioxin effect. We conclude that there is no evidence in these data that dioxin is adversely associated with birth defect severity.

12.11 Selected Birth Defects

Twelve birth defects (anencephaly, spina bifida, hydrocephalus, cleft palate, cleft lip/palate, esophageal atresia, anorectal atresia, polydactyly, limb reduction deformities, hypospadias, congenital hip dislocation, Down's syndrome) and 4 developmental anomalies (disturbance of emotion, hyperkinetic syndrome of childhood, specific delays in development, mental retardation) were investigated. Of these, there were only enough occurrences of specific delays in development and hyperkinetic syndrome of childhood to permit statistical assessment of associations with dioxin.

Pre-post SEA analyses without adjustment for covariates were carried out with Models 1, 2 and 3, first on all children and then with restriction to full siblings. Post-SEA analyses using the same models were also carried out on all children and then with restriction to full siblings. Each of the post-SEA analyses was carried out without and then with adjustment for covariates.

Pre-post SEA analyses of hyperkinetic syndrome of childhood were entirely negative. Unadjusted Model 1 and 2 analyses of specific delays in development found significant associations but these were not supportive of a hypothesis that dioxin is adversely associated with delays in development; the corresponding adjusted analyses were negative. The Model 1 findings were caused by a reversal in pre-SEA and post-SEA trends; the pre-SEA trends were increasing and the post-SEA trends were decreasing or not increasing with dioxin. The Model 2 finding was caused by high post-SEA rates of delays in development in children of Ranch Hands with intermediate dioxin levels and low rates in children of Ranch Hands with high dioxin levels. These patterns are not consistent with the expected dose-response and are inconsistent with each other and are therefore most likely chance occurrences.

Analyses of post-SEA hyperkinetic syndrome of childhood found one significant association in an adjusted Model 1 analysis restricted to full siblings. This finding was caused by a decreasing rate of hyperkinetic syndrome with dioxin in children of Ranch Hands. This finding is opposite to the expected dose-response and is most likely due to chance.

Analyses of post-SEA specific delays in development found one significant association in an adjusted Model 3 analysis. This finding was caused by the rate of delays in development being higher in children of Ranch Hands in the Low dioxin category than in children of Comparisons in the Background category. The rate in children of Ranch Hands in the High dioxin category was not significantly different from the rate in children of Comparisons in the Background category.

A significant interaction with the father's age at the time of birth of the child and the time of conception was found in a Model 3 analysis of specific delays in development. This interaction was caused by a significantly elevated rate in children of Ranch Hands in the Low dioxin category older than 30 years of age with the time of conception less than or equal to 4 years since the father's departure from SEA as compared with the rate in corresponding children of Comparisons in the Background category. Analyses within the other 3 strata determined by the father's age and the time of conception were negative.

These findings are weak, inconsistent and sometimes opposite to the expected dose response. They are therefore not supportive of a hypothesis of an adverse association between dioxin and delays in development or hyperkinetic syndrome.

12.12 Multiple Birth Defects

Of 1772 post-SEA children included in these analyses, 57 had multiple defects that could not be attributed to syndromes. Model 1 and 2 analyses of these 57 multiple birth defects found no significant associations with dioxin. Model 3 analyses found increased rates of multiple birth defects in children of Ranch Hands in the Low category relative to children of Comparisons in the background category. However, the rates in children of Ranch Hands in the High category were not significantly elevated. These findings are weak and inconsistent with the expected dose-response. We conclude that there is no evidence in these data that dioxin is adversely associated with multiple birth defects.

12.13 Neonatal and Infant Mortality

Analyses of post-SEA infant death were either negative or could not be carried out due to insufficient data. Analyses of post-SEA neonatal death found significant associations in two Model 3 analyses. Both of these findings were caused by the rate of post-SEA neonatal death being higher in children of Ranch Hands in the High dioxin category than in children of Comparisons in the Background category. However, the corresponding pre-post SEA data show that pre-SEA children of Ranch Hands in the High category also had a higher rate then pre-SEA children in the Background category. Therefore, these finding appear attributable to chance rather than to dioxin.

These analyses were generally negative and the two significant associations found in analyses of neonatal death were chance occurrences. We conclude that there is no association between dioxin and infant or neonatal mortality.

12.14 Summary

This study was motivated by significant variation at baseline in the rates of birth defects reported by spouses and partners of study participants. The Ranch Hand rate was lower than the Comparison rate in children born prior to the father's military service in SEA and greater than the Comparison rate in children born after the father's service in SEA, a finding that some interpret as suggestive of an adverse effect of dioxin on reproductive outcome. However, its interpretation as a dioxin effect was subject to objection because (1) the analysis grouped all defects together rather than by category, (2) the analysis was based on reported rather than verified birth defects and (3) because no account was taken of individual paternal dioxin levels.

Verification of all live births and conceptions reveal that Ranch Hands and Comparisons over- and under-reported birth defects similarly. About 2% of pre-SEA and post-SEA children were over-reported by both Ranch Hands and Comparisons. Both groups under-reported 14% of post-SEA defects and about 7% of pre-SEA defects.

A repetition of the baseline analysis using verified data derived from doctor and hospital records showed that the original finding remained. Subsequent analyses within each of 13 CDC categories of anomaly were carried out. Analyses in all categories except total congenital and nervous system were negative. The association in the nervous system category was in the same direction as that of total congenital, but there were too few nervous system anomalies (9 in Ranch Hand children and 10 in Comparison children) to attribute the overall result to anomalies of the nervous system.

Pre-post analyses of anomalies in each of the 13 categories were carried out using Models 1, 2 and 3 to assess the association between changes in rates and dioxin. No consistent or plausible evidence was found in any category of birth defect relating dioxin to a pre-post SEA reversal in rates. Thus, the baseline finding is still present in these data, but it is not related to dioxin. Additionally, the finding cannot be fully ascribed to any category of anomaly.

The lack of significant association between dioxin and the number of conceptions and between dioxin and any considered semen characteristic suggests that Ranch Hand dioxin exposure is unrelated to their ability to father children. The lack of consistent associations between dioxin and miscarriage, total adverse outcome, birth weight, any of 13 categories of birth defects and neonatal death suggests that dioxin is unrelated to reproductive outcomes fathered by this exposed population.

This study was based on reproductive outcome data verified from medical records up to the age of 18. In contrast, the CDC study [15] is based on data derived from parental report and birth records. Hence, some of our birth defect rates were higher than those reported by the CDC. For example, the crude rate of total congenital anomalies in post-SEA Comparison children is 266.9 per 1000 and the rate in Army Vietnam veterans derived from parental report is 64.6 per 1000. The two studies are comparable with regard to rates of specific defects based on verified data. For example, the rate of nervous system anomalies in post-SEA Comparison children is 1.5 per 1000 and the corresponding rate in post-SEA children of Army Vietnam veterans is 2.6 per 1000. The miscarriage rates in the two studies are similar. The miscarriage rate among post-SEA conceptions of Ranch Hands and Comparisons included in these exposure analyses is 145.5 per 1000; the miscarriage rate in post-SEA conceptions of Army veterans is 104 per 1000.

This and the CDC studies are generally negative. However, the CDC study found an association between service in Vietnam and nervous system anomalies; all analyses of nervous system anomalies except a pre-post SEA finding which was unadjusted for dioxin level, were negative in this study.

In any epidemiologic study, the possibility that bias in design or conduct may have affected the results need to be considered. It is unlikely that these results are biased due to differential reporting because the existence and contents of birth certificates, newborn clinic records or death certificates cannot be influenced by the parents. Additionally, these data were collected and verified at a time when the fathers had not yet received their dioxin results. However, we were concerned that Ranch Hand parents may have actively sought medical opinion regarding birth defects in their children, making birth defects more verifiable in their children than in Comparison children. We found no evidence of this 'verification' bias. We also investigated selection bias for the dioxin assay and found that children of enlisted ground personnel who volunteered for the assay were more likely to have birth defects than children of enlisted ground personnel who were not assayed. This selection bias is not detrimental to this report, however, because the birth defect rate was higher in children of assayed fathers than in children of unassayed fathers.

The many endpoints and multiple analyses in this report increase the likelihood that significant results will be found. In fact, if there is no relationship between dioxin and reproductive outcome, about 5% of the tests should be expected to produce significant results (p-values less than 0.05). The occurrence of significant results when there is no true effect is called the multiple testing artifact and is common to all large studies. There is no statistical procedure than can distinguish between artifactual results and those which are due to a true effect. Instead, we have relied on consistency and strength of association to interpret these findings. Based on these criteria we have concluded that all of the findings in this report are artifactual.

The few positive associations found between dioxin and the reproductive outcomes study in this report were generally weak, inconsistent or biologically implausible. These data provide no support for the theory that dioxin is adversely associated with reproductive outcome.

Whether dioxin exposure of the mother before or during pregnancy results in abnormalities in the developing fetus or child could not be addressed in this study and remains an open question.

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